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HUMAN DISEASE DUE TO AN 'AVIAN INFLUENZA' VIRUS: THE INFLUENZA A (H5N1) VIRUS

Influenza is caused by viruses belonging to the family orthomyxoviridae. Influenza viruses are classified into 3 types based on their core proteins: A, B and C. Influenza A viruses affect both humans and animals (including birds), B viruses affect humans only and C viruses affect humans and pigs only[1].

Influenza A viruses are further divided into several subtypes based on the haemagglutinin and neuraminidase proteins (surface proteins) present on the surface of the virus. There are 15 kinds of haemagglutinin (or 'H') protein, named from H1 to H15, and 9 kinds of neuraminidase (or 'N') protein, named from N1 to N9. No subtype exists for influenza B and C viruses[2].

Subtypes of influenza A virus demonstrate species-specificity. Thus those which infect animals do not usually cause infection in human beings. Influenza occurring in birds is called avian influenza (synonyms: bird flu, avian flu, fowl plague) and is caused by bird-species-specific subtypes of influenza A virus.

All the H subtypes *i.e.* H1 to H15 infect birds whereas only H1, H2 and H3 infect human beings. However, there are three subtypes which although usually infect birds but have also caused disease in humans. These are H5, H7 and H9. This was first reported in 1997 in Hong Kong when influenza A (H5N1) infection resulted in 18 cases with 6 deaths. Since then, many reports have appeared about bird-species-specific subtypes causing disease in humans (Table I). Recently, cases of human disease due to influenza A (H5N1) virus have been reported from Vietnam and Thailand also.

Table I. Outbreaks of human disease due to avian influenza viruses uptil March 2004[2,3].

Year	Virus	Place	No. of cases	No. of deaths
1997	H5N1	Hong Kong	18	6
1999	H9N2	Hong Kong	2	0
2003	H5N1	Hong Kong/ China	2	1
2003	H7N7	Netherlands	84	1
2003	H9N2	Hong Kong	1	0
2004	H5N1	Vietnam	22	15
2004	H5N1	Thailand	12	8

Most of the outbreaks described in table I have been associated with outbreaks of avian influenza in poultry caused by the same virus subtype (Table II).

If human disease due to influenza A (H5N1) continues to occur, a new pandemic *ie.* a global epidemic is likely to

Table II. Outbreaks of avian influenza in poultry coinciding with outbreaks of human disease due to avian influenza viruses (as of February 18, 2004)[3]

Year	Virus	Place	Type of bird	Mortality
1997	H5N1	Hong Kong	Chickens	1.5 million destroyed
2003	H7N7	Netherlands	Chickens	30 million destroyed
2004	H5N1	Vietnam	Ducks	27 million died or destroyed (as of February 18, 2004)
2004	H5N1	Thailand	Chickens, Ducks, Geese, Turkeys	27 million died or destroyed (as of February 18, 2004)

erupt because the current world population does not have immunity to this new virus subtype. (Some individuals are immune to the human subtypes H1N1, H2N2 and H3N2 due to past disease or immunization). The mechanism of creation of a pandemic strain is as follows:

The human influenza A viruses (H1N1), (H2N2) and (H3N2) as well as the avian influenza virus A (H5N1) possess a single strand of RNA consisting of 8 segments: PB2, PB1, PA, HA, NP, NA, 'M1 & M2' and 'NS1 & NS2'. This segmented genome may lead to genetic reassortment when the human respiratory epithelial cell is infected by viruses from two different species *eg*. a human virus and an avian virus. The human virus, to which humans are already prone, would now have acquired some avian genes, one of which may be the gene coding for the haemagglutinin (H) surface protein (H surface protein helps in binding of the virus to human cells). This would be the beginning of a new pandemic[4].

Genetic reassortment of human and avian strains is not new. First time this occurred in 1957. The H2N2 strain which caused the 1957 pandemic (the Asian flu) resulting in 1 million deaths possessed 5 genes from the preceding human H1N1 strain (PB2, PA, NP, 'M1and M2', 'NS1 and NS2') and 3 genes from an avian strain (HA, NA, PB1)[4].

Second time this occurred in 1968. The H3N2 strain which caused the 1968 pandemic (the Hong Kong flu), also resulting in 1 million deaths, possessed 6 genes from the preceding human H2N2 strain (PB2, PA, NP, 'M1 & M2', 'NS1 & NS2' and NA) and 2 genes from an avian strain (HA and PB1)[4].

Clinical Features of Influenza A(H5N1) Disease in Humans

Influenza A (H5N1) cases are characterized by high fever (above 100.4°F), cough and shortness of breath with the latter two developing early in the course of the disease. Clinically apparent pneumonia is seen alongwith chest X-ray changes (non-specific and may include diffuse, multifocal or patchy infiltrates and segmental or lobular consolidation with air bronchograms). The illness rapidly progresses to respiratory distress and subsequent respiratory failure within 1 week of onset of symptoms. Most cases die in spite of ventilatory support.[5]

Common laboratory findings are lymphopenia ($< 1x10^9$/litre) and slightly or moderately-raised alanine aminotransferase and aspartate transaminase[5].

The median time between exposure and onset of illness is 3 days (range 2 to 4 days). Any type of influenza tends to be more serious in children, elderly persons above 65 years of age and the chronically sick persons.

Children upto12 years of age are infective from 1 day before upto 21 days after the onset of symptoms. However, adults and children more than 12 years of age are infective from 1 day before the onset of symptom and upto 7 days after the resolution of fever.[6]

Workers handling poultry in farms, markets and involved in culling activity, veterinary workers and health workers are at higher risk of acquiring the infection. Even the family members of these workers are at high risk.

Clinical Features of Avian Influenza

The range of symptoms in birds varies greatly depending on the strain of virus and the type of bird. Clinical signs may include ruffled feathers, soft shelled eggs, depression and droopiness, sudden drop in egg production, loss of appetite, cyanosis, diarrhoea, blood-tinged discharge from nostrils, in-coordination including loss of ability to stand and walk, pin-point haemorrhages, respiratory distress and increased death losses in a flock.

Migratory waterfowl, most notably wild ducks, are the natural reservoir of all avian influenza viruses including H5N1. The virus multiplies in the epithelial lining of the intestinal and respiratory tracts of the affected birds and are excreted in high concentrations in faecal material. The waterfowl themselves develop very mild disease. However, domestic birds who come in contact with the faecal droppings of infected waterfowl can develop disease on a large-scale which is rapidly fatal.[8]

Influenza A H5N1 virus can survive at cool temperatures in contaminated manure for at least 3 months. One gram of contaminated manure contains enough virus to infect 1 million birds. The virus can survive in water for upto 4 days at 22°C and for >30 days at 0°C. The virus is killed by heat (56°C for 3 hours or 60°C for 30 minutes) and common disinfectants such as formalin and iodine compounds.[7]

Transmission of the Influenza A H5N1 Virus in Birds

Transmission from Infected Wild/Domestic Birds to Healthy Domestic Birds

Transmission from infected to healthy birds can occur by three methods.

Direct transmission

Direct transmission occurs immediately and without any intermediary. This takes place in two ways:

Direct contact

This referes to transfer of virus through contact of the nasal, oral or conjunctival mucous membranes of the infected birds with that of healthy birds. This can occur during mingling of infected wild or domestic flocks with healthy domestic flocks as occurs in non-enclosed poultry or household farms located close to water bodies visited by wild birds or in live markets. The virus present on the nasal mucous membrane surface or in nasal secretions; oral mucous membrane surface or in oral secretions, saliva, respiratory secretions and sputum; conjunctival mucous membrane surface or in ocular secretions of infected birds enter healthy domestic birds through nostrils, mouth and eyes.

Direct projection (or Droplet spread)

This referes to transfer of virus through droplet spray emitted from nostrils or mouth during coughing or sound production by infected birds. Droplets are particles which consist of viruses enclosed in respiratory secretions or salivary fluid and are more than 5 microns in size.

Indirect transmission

Indirect transfer of the virus from an infected to a healthy domestic bird takes place through following intermediary means.

Water-borne transmission

This occurs mainly by consumption of water contaminated by droppings or secretions of infected birds. The contamination can be with nasal secretions, oral secretions, saliva, respiratory secretions or sputum and ocular secretions. The contamination can take place directly by the infected bird or through flies or rodents whose feet or bodies have been contaminated with faeces or secretions of infected birds which in turn will contaminate the water.

Air-borne transmission

This occurs through dissemination of particles upto 5 micron in size consisting wholly or partially of viruses. These particles remain suspended in the air for long periods (Particles more than 5 micron in size cannot be transmitted by air, instead, they are involved in direct projection). Air-borne transmission is of two types:

Droplet nuclei : These are the residues that result from evaporation of fluid 'droplets' of nasal and oral secretions, saliva, respiratory secretions or sputum which contained the virus.

Dust : The virus is able to survive under certain temperatures in the contaminated soil and therefore in dust also, which is actually dried-up soil. The virus laden dust entering in the healthy birds through nostrils, mouth or eyes is an efficient vehicle for virus transmission.

Transmission of Influenza A H5N1 Virus in Humans[2-10]

Transmission of HSN1 avian influenza virus to healthy human beings from infected birds or human to human transfer is similar to that of bird to bird transfer of virus. In addition, human to human transfer of virus can also take place through equipments, instruments and other articles contaminated with secretions of infected persons. The route of entry of virus in human body is through mouth and nasal openings. A new virus adapted for efficient human-to-human transmission would spread very rapidly. There is no definite evidence of human to human transmission in the current episode.

Spread within a Country

The disease spreads easily from one poultry farm to another. Large amounts of viruses are secreted in bird droppings, contaminating dust and soil. Air-borne viruses can spread from bird to bird causing infection when the virus is inhaled. Contaminated equipments, vehicles, feed, cages or clothing, especially shoes can carry the virus from one farm to another. The virus can also be carried on the feet and bodies of animals, such as rodents, which act as mechanical vectors for spreading the disease. Limited evidence suggests that flies can also act as mechanical vectors.

Droppings from infected wild birds can introduce the virus into both commercial and backyard poultry flocks. The risk that infection would be transmitted from wild birds to domestic poultry is greatest where domestic birds roam freely, share water supply with wild birds, or use water supply that might become contaminated by droppings from infected wild-bird carriers.

Spread From One Country to Another

The disease can spread from one country to another through international trade in live poultry. Migratory birds, including wild waterfowl, sea birds, and shore birds can also carry the virus for long distances and, in the past, have been implicated in the international spread of highly pathogenic avian influenza. Migratory waterfowl notably the wild ducks – are the natural reservoir of bird flu viruses, however, these birds are highly resistant to infection. They can carry the virus over great distances, excrete them in their droppings, yet develop only mild and short-lived illness.

Case Definitions[2]

Suspect Case of H5N1 Influenza A

Persons with acute respiratory illness, characterized by fever (temperature > 38°C) with cough and/or sore throat and one or more of the following:

(i) contact with a confirmed case of influenza A (H5N1) during the infectious period,

(ii) recent (less than 1 week) visit to a poultry farm in an area known to have outbreaks of HPAI (highly pathogenic avian influenza), or

(iii) worked in a laboratory that is processing samples from persons or animals suspected to be having HPAI infection.

Probable Case of H5N1 Influenza A

(i) Suspect case; and

(ii) limited laboratory evidence for influenza A (H5N1) such as positive immunofluorescence antibody (IFA) using H5 monoclonal antibodies.

Confirmed Case of H5N1 Influenza

Suspect or probable case and one or more of the following:

(i) positive viral culture for influenza A (H5N1).

(ii) positive PCR for influenza A (H5N1).

(ii) a 4-fold rise in H5N1-specific antibody titer.

Laboratory Diagnosis

Laboratory diagnosis of H5N1 depends upon the demonstration of the virus and/or a rising antibody titre. Following tests are available (kits for these are being developed and may be available soon) for the laboratory diagnosis of H5N1:

(i) Virus culture;

(ii) RT-PCR;

(iii) Immunofluorescence using monoclonal antibody to H5N1; and

(iv) Serological tests (ELISA) for detection of specific antibody.

Of these, virus culture can only be done in laboratories with infrastructure, skills and reagents for isolation of influenza virus and confirmation of H5N1 subtype. These facilities are available only in a limited number of laboratories.

Rapid Tests for Diagnosis of Influenza Type A

Commercial rapid diagnostic tests are available which can be used to detect different types of influenza viruses within 30 minutes. Confirmation of H5N1 subtype can be done only in a well equipped laboratory with all facilities and adequate biocontainment measures. As of now no commercial kit is available which can diagnose infection due to H5N1 subtype.

General Biosafety Measures for Collection of Specimens from Suspected Cases of Avian Influenza

Clinical samples should be collected by trained hospital staff and not by the laboratory staff. The following precautions should be taken while collecting the clinical samples:

- Use N95 masks while taking samples. If not available, triple layer well fitted surgical face masks can be used.
- Use disposable latex gloves.
- Wear laboratory coat/disposable apron.
- Cover hair with head cover.
- Use protective eyewear (goggles)/face shields if procedure is likely to generate aerosols or splashes of secretions.
- Handle waste with special precautions while collecting specimens. The waste should be placed in an appropriate leak proof and autoclavable biohazard bag and autoclaved before disposal. Contaminated non-disposable waste should be treated properly.

- The clinical samples should be processed only in designated laboratory having the appropriate containment facilities.

Treatment

For majority of people, influenza is an upper respiratory tract infection that lasts several days and requires only symptomatic treatment and the antibiotics have no role to play. Severe cases of influenza would require:

- Admission to hospital;
- Intensive care;
- Antibiotic therapy to prevent secondary infection; and
- Ventilator for breathing support.

Aspirin is not given to children and teenagers to prevent occurrence of Reye syndrome. For several years, amantadine and rimantadine were the only antiviral drugs. However, while these drugs are relatively inexpensive and effective against type A influenza, they may be associated with severe side effects. When used for prophylaxis of pandemic influenza at lower doses, adverse events are far less. In addition, development of resistance by the virus against these drugs have already been reported. A new class of antiviral drugs, the neuraminidase inhibitors, has also been developed. It includes drugs like zanamivir and seltamivir. Though expensive, these have fewer adverse side effects and the virus less often develops resistance.

Prevention and Control

Several measures can help minimize the global public health risks that could arise from large outbreaks of highly pathogenic H5N1 avian influenza in birds. An immediate priority is to halt further spread of epidemics in poultry populations. This strategy works to reduce opportunities for human exposure to the virus. Vaccination of persons at high risk of exposure to infected poultry, using existing vaccines effective against currently circulating human influenza strains, can reduce the likelihood of co-infection of humans with avian and human influenza strains, thus minimising the risk that genes will be exchanged. Workers involved in the culling of poultry flocks must be protected against infection by proper clothing and equipment. These workers should also receive antiviral drugs as a prophylactic measures.

When cases of avian influenza in humans occur, information on the extent of influenza infection in animals as well as humans and on circulating influenza viruses is urgently needed to aid the assessment of risks to public health and to guide the best protective measures. Thorough investigation of each case is also essential. While WHO and the members of its global influenza network, together with other international agencies, can assist with many of these activities, the successful containment of public health risks also depends on the epidemiological and laboratory capacity of affected countries and the adequacy of surveillance systems already in place.

While all these activities can reduce the likelihood of emergence of a pandemic strain, the question of whether another influenza pandemic can be averted can not be answered with certainty.

Precautions for People Living in an Area having Outbreak of influenza due to H5N1 in Poultry[9,10]

Precautions for Persons Engaged in Raising Poultry

Whenever have contact with poultry, slaughter poultry, dispose of dead poultry, clean up the poultry's living areas or have contact with anything having poultry faeces on it, wear the following and do not involve children in these activities:

(i) Mask (If not possible, use a cloth around the mouth and nose).

(ii) Goggles.

(iii) Gloves (If not possible, use plastic bags to cover the hands),

(iv) Rubber boots (If not possible, use plastic bags to cover the shoes).

(v) Gown (If not possible, wear overalls that can be washed).

After finishing the job, remove all the protective apparel; discard the disposable materials and clean all reusable items; wash your hands, take a shower; and wash your clothes in hot, soapy water and hang them to dry in the sun.

Precautions for Persons Engaged in Culling of Poultry

(i) Wear the protective apparel as described above.

(ii) Collect any faeces which is scattered in the poultry living areas. (Keep sweeping to a minimum to prevent dust-borne transmission).

(iii) Bury the faeces at a depth of at least 2.5 metres.

(iv) After the job is over, carry out the post-activity steps as described earlier.

Precautions for Persons Visiting a Patient with H5N1 Influenza

(i) Avoid visiting during the period of communicability *ie*. upto 7 days after resolution of fever in adults and children <12 years old and upto 21 days after onset of illness in children ≥12 years of age.

(ii) If the visit can not be avoided during this period, one must wear mask (Fitted correctly), goggles, gloves and gown.

(iii) The protective apparal must be removed while leaving the room and hands should be washed thoroughly.

Precautions for Persons having Contact with a Patient during the Period of Communicability Without Wearing Protective Apparel

(i) Monitor your temperature for 7 days.

(ii) In case of fever, seek advice on chemoprophylaxis with antiviral medication.

(iii) If condition deteriorates, seek medical advice immediately and inform that you had contact with a case of H5N1 influenza.

Persons having contact with sick/dead poultry or faeces of these poultry without protective devices should monitor their temperature for 7 days and seek medical advice accordingly.

General Advice

(i) Persons with respiratory illness should cover their nose and mouth while coughing or sneezing;

(ii) One should always wash hands after touching any respiratory secretion; and

(iii) During illness one should always avoid being in the presence of small children or elderly people until symptoms have resolved.

Conclusions

Human disease due to H5N1 influenza A virus has never occurred in India earlier. However, because of it's geographical proximity to the affected countries and due to faster modes of travel such as air travel, there is always a risk of the disease occurring in India. Hence, there is a great need for surveillance for the disease through identification of the circulating strains of influenza virus in the country as well as preparedness for both laboratory identification as well as treatment and prevention modalities in case the disease occurs in the country.

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